REVIEW

Synergism between resveratrol and other phytochemicals: Implications for obesity and osteoporosis

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Resveratrol, a phytoalexin, has gained much attention recently due to its effects on sirtuins. While the anti-cancer properties of resveratrol have been extensively investigated, the anti-adipogenic and osteogenic effects of resveratrol are also gaining considerable interest. The finding that resveratrol supplementation mimics caloric restriction prompted researchers to study the effects of resveratrol on lipid metabolism. Mesenchymal stem cells are the precursors for both adipocytes and osteoblasts. In the aging population, differentiation to adipocytes dominates over the differentiation to osteoblasts in bone marrow, contributing to the increased tendency for fractures to occur in the elderly. Thus, an inverse relationship exists between adipocytes and osteoblasts in the bone marrow. Resveratrol acts on several molecular targets in adipocytes and osteoblasts leading to a decrease in adipocyte number and size and an increase in osteogenesis. Furthermore, resveratrol in combination with genistein and quercetin synergistically decreased adipogenesis in murine and human adipocytes. A recent in vivo study showed that phytochemicals including resveratrol in combination with vitamin D prevented weight gain and bone loss in a postmenopausal rat model. Therefore, combinations of resveratrol with other phytochemicals may lead to potential novel potent therapies for both obesity and osteoporosis.

Keywords:
Adipocyte life cycle / Multifocal signal modulation therapy / Osteogenesis / Synergistic activity

1 Introduction: Diet restriction, obesity and resveratrol

Dietary (caloric) restriction has been shown to positively influence the longevity of a range of animals including primates [1]. While this effect of resveratrol (3,5,4′-trihydroxystilbene), a polyphenol compound found in grapes, cranberries and many other plant species (Fig. 1), has been known for some time, the biochemical mechanisms were not well understood. Yeast cultures deprived of nutrients increase the activation of stress pathways, thereby deriving energy from alternative substrates, which results in a marked increase in the replicative lifespan (the number of daughter cells an individual mother cell produces before dying) [2]. This altered metabolism increases oxygen consumption, which causes changes in the ratio of oxidized to reduced forms of nicotinamide adenine dinucleotide (NAD/NADH). The increased NAD concentrations stimulate the activity of Sir2 (Silent Information Regulator, sirtuin protein). Sir2, the mammalian homologue of which is known as Sirt1, was shown to modify several proteins that are involved in cellular processes affecting longevity. It was shown that adding additional copies of the gene coding for

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Abbreviations: AMPK, AMP-activated protein kinase; **MSCs,** mesenchymal stem cells; **PPARγ,** peroxisome proliferator activated receptor γ; **PGC-1α,** peroxisome proliferator activated receptor γ coactivator-1 α; **RANKL,** receptor activator of NF-κB ligand; **Sir2, SIRT-2,** silent information regulator; **SREBP-1c,** sterol regulatory element binding protein 1c; **UCP-1,** uncoupling protein-1

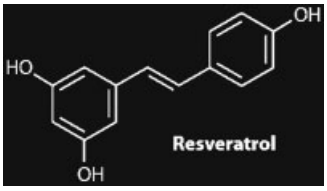

Figure 1. Chemical structure of resveratrol.

the production of Sir2 increased the life span of yeast and *Caenorhabditis elegans* [3, 4]. Interestingly, it was later shown that several polyphenols that directly or indirectly stimulate Sir2 activity also resulted in prolonged lifespan of yeast cells much the same way caloric restriction did [5]. Resveratrol was found to be one of the most active of these polyphenols. The yeast lifespan was increased from 21 for the untreated control cultures to 36 for the cultures treated with 100 μM resveratrol [5].

Over the past several decades an observation was made, coined as 'The French Paradox', that Frenchmen suffer a relatively low incidence of coronary heart disease, despite having a diet relatively rich in saturated fats. It has been suggested that the Frenchman's high red wine consumption is a primary factor for the trend. One of the components of red wine potentially related to this effect is resveratrol. When a description of this paradox was aired in the United States on 60 Minutes in 1991 with the proposal that red wine decreases the incidence of cardiac diseases, the consumption of red wine increased 44% and some wineries began lobbying for the right to label their products 'health food'. More recently, Baur et al. [6] showed that high doses of resveratrol mimicked some of the benefits of caloric restriction (including reduced effects of aging) in mice fed a high fat diet but not in mice fed a control diet.

These discoveries, especially triggered by the better understanding of the mechanisms of action of resveratrol on several diseases associated with aging, have led to new biotargets for drug discovery. The significant level of commercial funding has led to many new opportunities for novel strategies for drug discovery and therapies for diseases associated with aging. Numerous clinical trials are underway that are based on these discoveries. There has been much interest in resveratrol because it is an orally active phytochemical that, most likely at high daily doses, has many beneficial actions in a variety of animal disease models. The following is a summary of research on resveratrol's effects on the lifecycle of adipocytes and, thus, obesity and osteoporosis. The discussion includes also a summary of the strategy of discovering combinations of resveratrol with other phytochemicals that demonstrate synergies for beneficially impacting the lifecycle of adipocytes for the prevention and treatment of obesity and osteoporosis. The expectation is that carefully selected and formulated phytochemical combinations will make possible the use of phytochemicals at much reduced doses for the prevention and treatment of diseases associated with too many adipocytes filled with too much lipid, e.g. obesity, osteoporosis.

2 Osteoporosis and obesity: Socioeconomic impacts

Obesity and osteoporosis are major public health concerns due to their prevalence in our increasingly sedentary and aging society. In the USA today, an estimated 55% of people 50 and older are at risk for developing osteoporosis. Because as much as 20% of bone mass can be lost in the first 5–7 years following menopause, osteoporosis is a major health issue for aging women. Eighty percent of those diagnosed with osteoporosis are female, and in women the risk of hip fracture due to osteoporosis is equal to the combined risk of breast, uterine and ovarian cancer [7]. Approximately 24% of women over the age of 50 who sustain a hip fracture die in the year following their fracture, and 20% of those who were ambulatory before their hip fracture required long-term care afterward [7]. In 2005, osteoporosis-related fractures were responsible for an estimated $19 billion in costs. By 2025, these costs are predicted to rise to approximately $25.3 billion, and over the next 50 years, the national cost may be as high as $240 billion [7].

Worldwide over 1 billion adults are overweight, with more than 300 million clinically obese. The latest data from the National Health and Nutrition Examination Survey (NHANES) indicated that in the USA by 2008 the overall prevalence of obesity was 32.2% for adult men and 35.5% for adult women, while the combined prevalence for obesity and overweight was 72.3% for men and 64.1% for women [8]. National data show that the prevalence of obesity has steadily increased over the past three decades. If these trends continue, in only 15 years 80% of all American adults will be overweight or obese, and by 2040 100% of adults are predicted to be overweight or obese [9].

3 Regulation of adipocyte and osteoblast growth and development

It was once believed that the number of adipocytes remained constant over one's life time and adipocytes can neither be gained nor lost. However, in the past decade researchers acknowledged that adipocytes can be both gained and lost, and the metabolic consequences of obesity depend on whether an increase in adipose tissue mass occurred due to hyperplasia of adipocytes or as a result of hypertrophy [10]. In bone marrow, adipocytes and osteoblasts are derived from a common progenitor cell, the mesenchymal stem cell (MSC). MSCs derived from adipose tissue are also a good source of bone-forming cells and adipose tissue derived MSCs are now being considered a source for cell therapy and tissue engineering applications owing to high yield and relative ease of obtaining cells compared with using bone

marrow as a source [11]. Recently, adipose-derived MSCs were used in the regenerative treatment of traumatic calvarial bone defect in humans [12].

The adipocyte life cycle starts with differentiation of adipocytes either from embryonic stem cells or MSCs (Fig. 2). Gregoire et al. [13] listed the events in adipocyte differentiation as growth phase followed by growth arrest, clonal expansion, complex sequence of changes in gene expression leading to lipid storage and finally cell death. Rayalam et al. illustrated various stages in the life cycle of adipocytes starting from preadipocytes, maturing preadipocytes and mature adipocytes and suggested possible ways to influence adipocyte number and size [14] by interfering with adipocyte life cycle. Adipocytes can be eliminated by inducing apoptosis, a process of programmed cell death, in pre-, maturing or mature adipocytes, or adipocytes can be gained by inducing preadipocyte proliferation. Likewise, adipocyte size can be altered either by increasing/decreasing lipogenesis or by stimulating or suppressing lipolysis [14]. Phytochemicals like resveratrol, genistein, guggulsterone and xanthohumol have been shown to affect the adipocyte life cycle at various stages [15, 16].

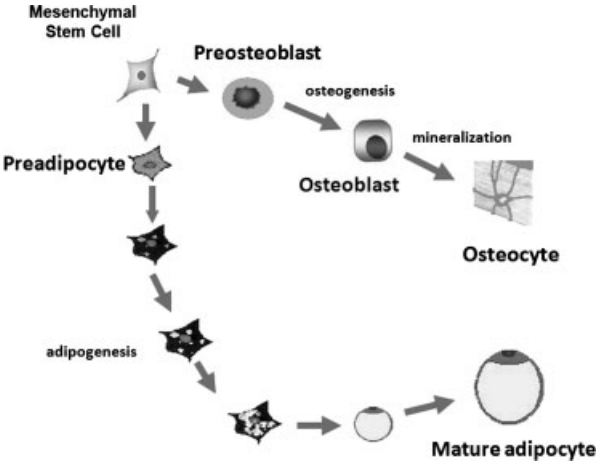

Figure 2. Adipocyte and osteoblast life cycle. Mesenchymal stem cells are the precursors of both adipocytes and osteoblasts. While PPARγ is considered a master regulator of adipogenesis, it negatively regulates bone formation in bone marrow. Thus, a negative correlation exists between adipocytes and osteoblasts in bone marrow. Once preadipocytes are triggered to mature, they proliferate and undergo growth arrest followed by a round of cell division and commitment cells subsequently differentiate into mature adipocytes. This is accompanied by a dramatic increase in the expression of adipocyte-specific genes. Mature adipocytes can continue storing lipid when energy intake exceeds output, and they can mobilize lipid through lipolysis when energy output exceeds input. Mature adipocytes can also undergo apoptotic cell death under certain conditions. Conversion of preosteoblasts to osteoblasts is also accompanied by an increase in the expression of several osteogenic genes. Osteoblasts further produce osteocytes and are responsible for mineralization resulting in calcified bone formation.

Osteoblast differentiation from MSCs (Fig. 2) is also tightly regulated. Pei and Tontonoz [17] indicated that an insufficiency of peroxisome proliferator activated receptor γ (PPARγ), a key transcription factor, which is considered a master regulator of adipogenesis and is implicated in lipid metabolism, promotes osteoblast formation. Deletion of PPARγ resulted in an increased expression of key molecules for osteoblastic differentiation like Runx2 and osterix. Further, no change in the osteoclast functioning was noticed in cells lacking PPARγ [18]. Wnt signaling also diverts MSCs towards osteogenic lineage. While osteoblast precursors enhance bone resorption by stimulating receptor activator of NF-κB ligand (RANKL)-induced osteoclast activation, mature osteoblasts block RANKL-induced osteoclastogenesis by increasing osteoprotegerin expression [19]. Thus, in bone marrow a reciprocal relationship exists between the development of adipocytes and osteoblasts. This is further supported by the findings of Backesjo et al. [20, 21], who showed that the activation of Sirt1 decreased adipocyte formation and promoted osteoblast differentiation in bone marrow stromal cells and MSCs. Therefore, compounds that inhibit adipogenesis may have positive effects on bone formation, suggesting a potential research area for osteoporosis intervention.

4 In vitro effects of resveratrol in adipocytes

4.1 Anti-adipogenic and pro-apoptotic effects

The effect of resveratrol on adipogenesis is well researched. It has been shown to reduce the synthesis of lipids in 3T3-L1 adipocytes [22] and pig primary adipocytes [23]. In addition to decreasing lipid accumulation in maturing 3T3-L1 preadipocytes, resveratrol also decreased cell viability [24]. Decrease in adipocyte viability was due to induction of apoptosis by resveratrol. Anti-proliferative and apoptotic effects of resveratrol are well studied in various cell lines including cancer [25, 26]. Further, an analysis of PubMed database search for resveratrol and apoptosis shows over 600 articles, whereas search for resveratrol and adipogenesis shows fewer than 20 articles, indicating that anti-adipogenic effects of resveratrol have been of interest only recently.

Inhibition of proliferation and adipogenic differentiation by resveratrol occurs in a SIRT1-dependent manner in human preadipocytes [27]. On the contrary, resveratrol synergistically enhanced tumor necrosis factor-α-related apoptosis-inducing ligand (TRAIL)-induced apoptosis of human adipocytes, and this effect was independent of SIRT1 [28]. Furthermore, the antioxidant activity of resveratrol in preadipocytes caused a decrease in membrane potential resulting in an increased number of apoptotic but not necrotic cells associated with an increase in caspase-3 activity and downregulation of Bcl2 proteins [29]. Similarly, the anti-adipogenic effects of resveratrol are associated with

downregulation of several adipocyte-specific transcription factors like PPARγ, CCAAT-enhancer-binding protein α (C/EBPα) and sterol regulatory element binding protein 1c (SREBP-1c). In mature human adipocytes, resveratrol also increased basal and insulin-stimulated glucose uptake but inhibited lipogenesis in parallel with a downregulation of lipogenic gene expression [27].

4.2 Effects on lipolysis

Lipolytic effects of resveratrol are also driven by SIRT1 activation. Activation of SIRT1 by resveratrol mobilizes lipids from white adipocytes in vivo in rats and 3T3-L1 adipocytes in vitro. This is accomplished by the repression of PPARγ by docking of SIRT1 with cofactors of PPARγ, like nuclear receptor co-repressor and silencing mediator of retinoid and thyroid hormone receptors [22]. Resveratrol enhances cAMP levels in various cell lines, which is crucial for lipolysis [30]. Furthermore, in adipocytes resveratrol enhanced epinephrine-induced lipolysis [31].

4.3 Resveratrol and mitochondrial biogenesis

The anti-adipogenic effects of resveratrol are indirectly influenced by altering the expression of genes that modulate mitochondrial function. PPARγ coactivator-1α (PGC-1α), a mitochondrial development-related gene, plays an important role in regulating mitochondrial biogenesis and oxidative metabolism and maintaining the balance between glucose, lipid and energy [32, 33]. Resveratrol activates SIRT1, which further deacetylates the PGC-1α at promoter regions to induce the expression of genes involved in adipogenesis and fatty acid oxidation [34]. In addition, resveratrol increases the expression of mitofusin 2, a mitochondrial membrane protein that participates in mitochondrial fusion in mammalian cells and has been shown to play an important role in glucose oxidation in preadipocytes [24]. Uncoupling protein 1 (UCP-1), which resides on the mitochondrial inner membrane and mediates adaptive thermogenesis is also upregulated by resveratrol [24] (Fig. 1). In contrast, recent studies indicate that short-term treatment of adipocytes in vitro with resveratrol resulted in the attenuation of metabolic activity of mitochondria [35].

4.4 Anti-inflammatory effects

Anti-inflammatory effects of resveratrol have been described in several diseases like cancer [36], arthritis [37] and pancreatitis [38]. The anti-inflammatory effects of resveratrol in cancer are mediated via the inhibition of enzymes, cyclooxygenases and lipooxygenases that synthesize proinflammatory mediators from arachidonic acid [39]. Adipose tissue also produces proteins that are classical mediators of the inflammatory response. Pro-inflammatory cytokines like TNF-α, plasminogen activator inhibitor-1, interleukin-1β (IL-1β) and IL-6 are secreted by adipocytes, resulting in the enhanced systemic levels of these cytokines in obese subjects [40]. Resveratrol has been shown to have specific anti-inflammatory effects in adipocytes. The expression of TNFα, IL-6 and cyclooxygenase-2 is reduced by resveratrol in murine adipocytes [41]. Resveratrol also reversed the TNFα-induced secretion and mRNA expression of plasminogen activator inhibitor-1, IL-6 and adiponectin in 3T3-L1 adipocytes [42]. TNF-α mediates its effects on adipocytes by activating the NF-κB signaling pathway, and resveratrol is a potent inhibitor of NF-κB activation indirectly influencing the adipocyte differentiation [43]. In addition, resveratrol is a potent reactive oxygen species (ROS) scavenger [44] resulting in reduced oxidative stress, which contributes to suppression of inflammation. These inhibitory effects on inflammatory response suggest resveratrol as a novel anti-inflammatory agent for reducing several disease conditions associated with inflammation.

5 Anti-obesity effects of resveratrol in rodents

There is no definitive pharmaceutical cure for obesity, and currently available medications are not only unsuccessful in treating this disease but also are often associated with serious side effects. Phytochemicals provide huge potential in addressing complex diseases like obesity owing to their multiple targets. Implications for resveratrol in obesity have been discovered recently, and there are limited studies showing anti-obesity effects of resveratrol in vivo. A dietary supplementation of mice with 400 mg/kg/day in high fat diets increased their resistance to obesity by causing diminished total body fat content and decreasing depots of epididymal, inguinal and retroperitoneal white adipose tissue [45]. It is interesting to note that the body weight reduction in these animals was the result of less adipose tissue in the resveratrol high-fat-diet treated mice albeit no effect on food intake. As seen in the in vitro studies, resveratrol treatment induced marked mitochondrial morphological changes and also increased UCP-1 expression levels in brown adipose tissue. Resistance to weight gain in these animals is likely contributed by the effects of resveratrol on mitochondrial biogenesis leading to increased energy expenditure. These effects in turn are related to the increased activity of PGC-1α.

In contrast to the findings in the study of Lagouge et al., Baur et al. [6] demonstrated that resveratrol supplemented at a much lower dose of 22.4 mg/kg/day in a high fat diet over a course of 1 year did not modify weight gain. However, resveratrol improved insulin sensitivity and increased the survival rate in these mice. While both of these studies suggest an increase in PGC-1α deacetylation due to upregulated SIRT1 levels as the mechanism of action of resveratrol's

effects, Um et al. reported AMP-activated protein kinase (AMPK) as a key target for resveratrol [46].

In AMPKα1 knockout mice, resveratrol failed to diminish the high fat diet induced obesity and failed to increase insulin sensitivity, glucose tolerance and mitochondrial biogenesis. However, a link between SIRT1 and AMPK was later established, indicating that AMPK regulates energy expenditure by modulating SIRT1 activity [47]. Moreover, AMPK and SIRT1 affect acetylation status of transcriptional regulators such as the Forkhead box protein O (FOXO) family, resulting in the modulation of mitochondrial function and lipid metabolism [47, 48]. Interestingly, when aged mice were supplemented with resveratrol at doses that could be easily achieved in humans (4.9 mg/kg/day), it mimicked caloric restriction and reverted the gene-expression profiles associated with the aging of heart and skeletal muscle and prevented dysfunctions due to cardiac aging. Surprisingly, in this study resveratrol did not modify factors like SIRT1 and PGC-1α that have been previously suggested to explain its action [49].

In a recent study, SREBP-1c, a key lipogenic activator was reported as a major in vivo target for SIRT1. Treatment of mice with resveratrol for 1 wk decreased acetylated SREBP-1c levels associated with reduced lipogenic gene expression and fatty liver [50]. Authors suggest that resveratrol may also inhibit lipogenesis in vivo by reducing the acetylation of SREBP-1c via SIRT1 activation. Further, involvement of the central nervous system in resveratrol's effects is also reported. Long-term intracerebroventricular infusion of resveratrol normalized hyperglycemia and improved hyperinsulinemia in diet-induced obese and diabetic mice [51]. Although contrasting targets and results are reported on the effects of resveratrol on glucose metabolism, type-2 diabetes mellitus, obesity and aging, the data reported in the literature are lacking on the effect of the resveratrol at pharmacological concentrations attainable in vivo and, possibly, not too far from those achievable in the diet [52].

6 Osteogenic effects of resveratrol

Current therapies for osteoporosis act by either inhibiting osteoclast-mediated bone resorption or by promoting osteoblast-mediated bone formation. Recent studies show that resveratrol acts by both antagonizing osteoclast and promoting osteoblast differentiation in vitro [53]. There are multiple ways in which resveratrol might promote bone health: (i) resveratrol stimulates AMPK activation, and activated AMPK in turn stimulates the differentiation and proliferation of osteoblasts. AMPK also acts as a negative regulator of RANKL thereby inhibiting the resorption of bone that is stimulated under RANKL-activated conditions [54]. (ii) Activation of SIRT1 by resveratrol decreases adipocyte development via inhibition of PPARγ, which in turn promotes osteoblast differentiation [20]. (iii) Estrogenic effects of resveratrol via estrogen receptor activation coupled with extracellular signal-regulated kinase 1/2 (ERK 1/2) activation stimulates osteoblast proliferation and differentiation [55]. (iv) Resveratrol augments Wnt signaling, which plays an important role in promoting osteoblastogenesis and bone formation [56]. In addition, estrogenic effects of resveratrol through interaction with estrogen receptors α and β also contribute to its osteogenic effects [57]. There are limited studies demonstrating the osteogenic effects of resveratrol in vivo. In mice fed with 400 mg/kg feed of resveratrol, a significant improvement in bone mineral density as assessed with micro-computed tomography (micro-CT) and bone volume to total volume ratio was observed compared with mice fed with standard control diet, indicating that resveratrol may improve bone health [58].

7 Resveratrol in multifocal signal modulation therapy

Lack of success with targeted monotherapy has prompted researchers to explore targeting multiple targets simultaneously especially as an approach for cancer therapy. Numerous molecular targets for natural compounds have been identified in recent years. These are of two types, targets to which the natural compounds directly bind and modulate their activity, and targets that are modulated indirectly. While SIRT1 [6, 45] and AMPK [46] are reported as targets for resveratrol, it is not completely understood whether activation of these enzymes is a direct or indirect effect of resveratrol. On the other hand, owing to its structural similarity to 17 β-estradiol, resveratrol binds to both α and β estrogen receptor subtypes [57] and elicits estrogenic effects.

Pharmacokinetic studies indicated that extensive sulfation and glucuronidation of resveratrol in small intestine and liver as contributing factors affecting its bioavailability [59–61]. Interestingly, the phytoestrogen quercetin inhibits sulfation and glucuronidation of resveratrol in the duodenum and liver and thus may indirectly increase the bioavailability of resveratrol [60, 62]. Thus, combining multiple natural products may result in synergistic activity resulting both from increases in bioavailability and from actions on multiple molecular targets, thus offering several advantages over treatments with single compounds alone.

Resveratrol, in particular, when combined with other natural compounds caused enhanced effects on inducing adipocyte apoptosis and inhibiting adipogenesis. Genistein and quercetin have been studied in combination with resveratrol. Genistein is a soy isoflavone and is well known for its anti-adipogenic and lipolytic effects in vitro and in rodents [63, 64]. Quercetin, a flavonol, is found in many common foods, including black and green tea, apples and onions. In adipocytes, quercetin decreased viability, induced apoptosis and stimulated lipolysis [65, 66]. Genistein and resveratrol in combination exerted an enhanced effect in

3T3-L1 adipocytes on inhibiting adipogenesis, inducing lipolysis and triggering apoptosis [16]. Likewise, resveratrol and quercetin also acted synergistically in causing anti-adipogenic and pro-apoptotic effects in 3T3-L1 adipocytes [65]. While genistein, quercetin and resveratrol each produced anti-adipogenic activities in adipocytes, in combination they caused enhanced inhibition of lipid accumulation in both 3T3-L1 adipocytes and primary human adipocytes. A combination of these three phytochemicals further synergistically induced apoptosis in murine and human adipocytes, suggesting a potentiated effect in decreasing both adipocyte number and size [67].

8 Effects of resveratrol in combination with other phytochemicals in vivo

Although there are reports of synergy between resveratrol and other phytochemicals in vitro [67], there is no previous evidence for synergy in vivo. Recently, resveratrol in combination with vitamin D, genistein and quercetin was tested in aged ovariectomized rats, an animal model for postmenopausal bone loss. Rats supplemented 2400 IU/kg vitamin D, 400, 2000 and 1040 mg/kg diet of resveratrol, quercetin and genistein, respectively, had reduced weight gain and adiposity without a change in food intake, compared with both phytoestrogen-free control diet and control diet plus vitamin D alone [68]. In addition, the bone marrow adipocyte density was significantly reduced with an associated decrease in osteoclasts compared with both control and vitamin D treatments. Further, the micro-CT analysis of femur showed a marked increase in trabecular bone density with vitamin D plus phytochemicals compared with other two control groups. Even though this study lacked a resveratrol alone group, this is the first evidence for enhanced anti-obesity and pro-osteogenic effects in vivo with a combination of phytochemicals that include resveratrol. These results may be particularly relevant in the postmenopausal bone loss process since resveratrol in combination with other phytochemicals and vitamin D not only enhanced bone density but also decreased adipocyte numbers in bone marrow. It is known that an increase in adipocytes in bone marrow leads to an increase in adipocyte-derived factors that stimulate osteoclastogenesis leading to bone resorption [69, 70]. By modulating several signaling pathways simultaneously, resveratrol in combination with other phytochemicals may have potential to address complex diseases like obesity and osteoporosis (Fig. 3).

9 Toxicity of resveratrol

Resveratrol exerts dose-dependent biphasic effects in vitro and in vivo. At high doses in the rage of 10–40 mM, it acts as an apoptotic agent for cancer prevention, whereas at a relatively low dose of 5–20 μM, it acts as anti-apoptotic agent providing cardioprotection (reviewed in [71]). In contrast to

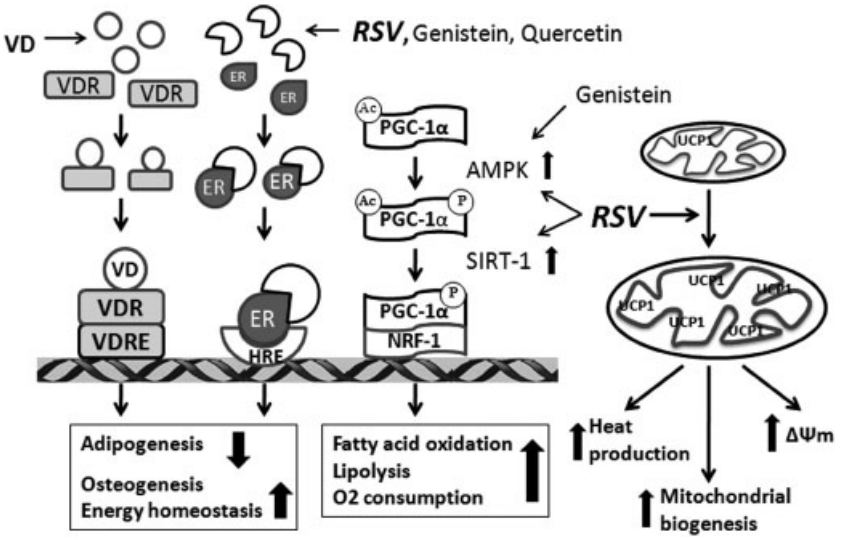

Figure 3. Synergistic effects of resveratrol in combination with vitamin D and other phytochemicals in adipocytes. Resveratrol not only activates AMPK/SIRT-1 but also increases UCP-1 expression in mitochondria causing increased energy expenditure in addition to increasing fatty acid oxidation and decreasing adipogenesis. When resveratrol is supplemented with other phytochemicals like genistein and quercetin and vitamin D, a synergistic decrease in adipogenesis, body weight gain and bone loss is observed in ovariectomized aged rats, which is likely a result of multiple pathways being triggered, as illustrated. AMPK, adenosine monophosphate kinase; ER, estrogen receptor; HRE, hormone response element; NRF-1, nuclear respiratory factor 1; PGC-1α, peroxisome proliferator activated receptor γ coactivator-1α; RSV, resveratrol; SIRT-1, sirtuin 1; UCP-1, uncoupling protein 1; VD, vitamin D; VDR, vitamin D receptor; VDRE, vitamin D responsive element; $\Delta\psi_m$, mitochondrial membrane potential.

the cardioprotective effects of resveratrol both in vitro and in vivo, oral administration of high dose (1 mg/kg) of resveratrol to hypercholesterolemic rabbits promoted atherosclerosis [72]. In a different study, potential toxicity of resveratrol was evaluated by gavaging 0.3, 1 and 3 g/kg feed to rats for 28 days and the results indicated that 3 g/kg dose of resveratrol caused renal toxicity, whereas 0.3 g/kg was not toxic [73]. In support of these findings, another study reported that a modest dose of resveratrol increased life span in mice while a larger dose of about 2 g/kg resulted in mortality [58]. Thus, the beneficial effects of resveratrol are dose-dependent. Another limitation for resveratrol use is its bioavailability. Following oral dosing, the bioavailability of resveratrol was only 20% in rats [74]. Furthermore, it is rapidly metabolized to glucuronide and sulfate conjugates and about 75% is excreted via feces and urine [75]. Conjugated resveratrol was found in the circulation predominantly compared with the free form, indicating that the metabolites of resveratrol may play an important role in its biological activity [75].

10 Summary

Epidemiological studies indicate that the prevalence of obesity and osteoporosis is rapidly increasing worldwide, and a recent study conducted by the Organization for Economic Cooperation and Development showed that three out of four Americans will be overweight by 2020. Under these circumstances, it seems preposterous to suggest three quarters of the population should be prescribed weight reduction medications. On the contrary, lifestyle changes accompanied by the intake of dietary supplements that promote bone growth and reduce weight gain are plausible recommendations. Phytochemicals have the potential to act on multiple targets and this approach is preferred to avoid compensatory mechanisms that occur with therapies with a single target, especially when addressing complex diseases like obesity and osteoporosis.

Resveratrol acts on multiple targets as reviewed in [76] to decrease adipocyte number and size and to promote bone formation. The decrease in adipose mass is mediated not only through downregulating adipocyte-specific transcription factors and enzymes but also by genes that modulate mitochondrial function. Further, these responses were shown to be greatly enhanced when resveratrol was combined with other phytochemicals both in vitro and in vivo. Being a potent activator of SIRT1/AMPK, resveratrol has attracted not only nutraceutical research resources but also pharmaceutical entities worldwide for its lifespan extension benefits. It should be noted that combining resveratrol with other phytochemicals may provide an extraordinary potential for preventing obesity and osteoporosis by not only decreasing the dose of each compound, thereby avoiding potential toxic side effects, but also by targeting multiple signaling pathways affecting adipogenesis, apoptosis, lipolysis and osteogenesis simultaneously. These phytochemical synergies may make possible novel safe, potent and efficacious therapies.

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